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OVIPOSITOR ULTRASTRUCTURE AND OVIPOSITION BEHAVIOR OF THE CRYPTIC AND SYMPATRIC SPECIES, *TRUPANEA NIGRICORNIS* (COQUILLETT), A POLYPHAGE, AND THE NARROWLY OLIGOPHAGOUS *T. BISETOSA* (COQUILLETT) (DIPTERA: TEPHRITIDAE)

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Abstract.—The flower head infesting tephritids, *Trupanea nigricornis* (Coquillett) and *T. bisetosa* (Coquillett) are cryptic and sympatric species. *Trupanea nigricornis* is a polyphagous species while *T. bisetosa* is a specialist on wild sunflowers. The two species showed major differences in their oviposition behavior. Females of *T. nigricornis* oviposited in various developmental stages of open immature heads of *Encelia* spp. and always pierced the plant tissues during oviposition; whereas, *T. bisetosa* females only oviposited in the early stages of closed buds of wild sunflowers and deposited their eggs loosely between the florets without injuring plant tissues. Timing of oviposition without plant tissue injury by *T. bisetosa* was critical because older buds were covered with hard bracts and exuded resin when injured. The period of flower head suitability for oviposition was shorter for *T. bisetosa* than *T. nigricornis*. The differences in oviposition behavior are reflected in the ultrastructure of their ovipositors. The aculeus tip of *T. nigricornis* is pointed, whereas that of *T. bisetosa* is rounded. The acanthae covering the ventral side of the eversible membrane have pointed tips in *T. nigricornis* and are rounded in *T. bisetosa*. *Trupanea nigricornis* has two pairs of central ampulliform sensilla at the apex of the aculeus while *T. bisetosa* has three pairs. Therefore, ovipositor morphology reflects oviposition behavior.

Key Words: Diptera, Tephritidae, *Trupanea*, oviposition behavior, ovipositor structure, acanthae, sensilla, phenology, *Encelia*, *Helianthus annuus*

The flower head infesting tephritids, *Trupanea nigricornis* (Coquillett) and *T. bisetosa* (Coquillett) occur in sympatry in southern California (Foote et al. 1993). They are closely related, cryptic species as they are similar morphologically and genetically, yet they do not interbreed (Knio et al. 1996a, 2007). The immature stages of both species are difficult to separate and are best identified according to their host plants (Knio et al. 1996a). The adults show great morphological similarities. Males can be distinguished by the color of their third antennal segment and most females by the shape of the Y-shaped apical marking on the wing (Cavender and Goeden 1983, Foote et al. 1993). Resource

utilization studies demonstrated that the larvae of both species exploited the flower heads of their host in a similar manner and fed on a relatively similar number of achenes (Knio et al. 2001). Nevertheless, the two sympatric species show major ecological differences. *Trupanea nigricornis* behaves as a polyphagous species infesting the heads of 33 genera belonging to at least 8 tribes of the Asteraceae, while *T. bisetosa* is narrowly oligophagous, attacking 6 species of the tribe Heliantheae, and mainly specializing on wild sunflowers in southern California (Goeden 1985, 1992).

Behaviorally, *Trupanea nigricornis* and *T. bisetosa* show a number of differences. Adult males differ subtly in courtship behavior and in the timing of mating in the field. Males of *T. nigricornis* were observed to court in the mornings whereas those of *T. bisetosa* exhibited courtship display in the afternoon (Knio et al. 1996b). On the other hand, females differed greatly in their oviposition behavior. *Trupanea nigricornis* females always pierced the plant tissues during oviposition, such that the posterior end of the egg was inserted into plant tissues. In most cases (ca. 80%), females deposited 1–3 eggs per flower head. On the other hand, *T. bisetosa* females never pierced or injured plant tissues during oviposition and the eggs were vertically aligned loosely atop or among the corollas. In most cases (ca. 70%), *T. bisetosa* females placed 3–8 eggs per flower head (Knio et al. 1996b).

Fecundity studies demonstrated that *T. nigricornis* females had a higher fecundity than *T. bisetosa*. In no-choice experiments, *T. bisetosa* did oviposit in the non-host flower heads of *Encelia farinosa* (Gray); however, *T. nigricornis* could not oviposit in the non-host flower heads of *Helianthus annuus* L. because of the morphological features of this plant (Knio et al., in press). These observations emphasize the importance of the differences in oviposition behavior between the two species and the differences in the ultrastructure of their ovipositors. Sensory structures on ovipositors play an important role in determining host plant suitability in tephritids (Schoonhoven 1983). Further, timing of oviposition by *T. bisetosa* females on their wild sunflower hosts is critical for overcoming the problem of the hard bracts and plant resins. Thus, host plant usage and specialization is not only determined by host plant chemistry, but by other factors like the biophysical features of the plant, the synchronization of adult emergence and female oviposition with host phenology, and availability of buds at stages suitable for egg laying (Berube 1978b, Straw 1991, Zwölfer and Harris 1971).

In this study, we examine the phenology of flower head development of the most common hosts of *T. nigricornis* and *T. bisetosa* in relation to oviposition suitability and we investigate whether the differences in ovipositor morphology relate to differences in oviposition behaviors. This paper is the last of a series aimed at shedding light on the nature of polyphagy/monophagy in closely related sympatric and cryptic tephritids.

Materials and Methods

Phenology of flower head development.—The phenology of flower head development of *Encelia farinosa* (Gray) (Asteraceae) and *Helianthus annuus* L. (Asteraceae), the most common hosts of *T. nigricornis* and *T. bisetosa*, respectively, in relation to oviposition was studied in the field. The sites were: University of California, Riverside Co., CA (site 1); Casa Blanca, Riverside Co., CA (site 2), and Lake Perris, Riverside Co., CA (site 3). Only site 3 was observed for *T. bisetosa*. At each location, 10–20 individual flower heads on different spikes were labeled with masking tape attached to the peduncle of each flower head. The

flower heads were in the earliest stage of development as 'unopened' buds. The terminal flower heads were not labeled because they developed and matured faster than the apical ones found on different peduncles of the same spike. Development of these labeled flower heads was followed in the field. Flower head diameters at their maximum width and lengths from the base of the receptacle to the tip of the florets were measured using dial calipers at days 1, 3, 5, 10, 15, 18, 22, 28, 33, and 40. At each of these intervals, the labeled flower heads were checked for oviposition wounds, and 20–40 other flower heads at the same developmental stage were collected and dissected in the laboratory to record the number of *T. nigricornis* or *T. bisetosa* eggs.

Insect rearing for scanning electron microscopy.—*Trupanea nigricornis* adults were reared from flower heads of *E. farinosa* while those of *T. bisetosa* were reared from wild sunflower heads, *H. annuus*. The mature flower heads, containing third instar larvae or puparia, were placed in glass-topped, sleeved insectary cages (34×32×35 cm) at the University of California, Riverside, at 60% RH and 12/12 (LD) photoperiod from 0500–1700 h.

Ovipositor ultrastructure.—The ovipositors of *T. nigricornis* females (n = 4) and *T. bisetosa* females (n = 3) were fixed in 2% gluteraldehyde for 12 h, washed twice in distilled water, post-fixed in 2% osmium tetroxide overnight, washed twice in distilled water, dehydrated in an increasing series of ethanol, then washed twice in absolute ethanol. The specimens were critically point-dried, mounted on stubs, and coated with a gold-platinum alloy before examination with a scanning electron microscope (SEM), at 15 kV accelerating voltage. Micrographs were taken using Polaroid 55P/N ® films. The micrograph negatives are stored with D. H. Headrick at California Polytechnic State University, San Luis Obispo.

The terminology used in the description of the ovipositor of *T. nigricornis* and *T. bisetosa* follows that of White et al. (1999).

Results and Discussion

Phenology of flower head development.—The development of *Encelia farinosa* flower heads was divided into the following stages: (1) 'closed bud' stage, in which the bracts cover the immature florets; (2) 'open, green bud' stage, in which the immature florets are exposed, but still green; (3) 'open, light –green bud' stage, in which the immature florets are light-green; (4) 'open, yellow bud' stage, in which the florets are turning yellow, but are not mature, and a few ray florets are starting to develop; (5) 'blossom' stage, in which the florets are at anthesis and the ray florets are fully developed; and (6) 'post-blossom' stage, in which the achenes are mature and hard, the florets begin to dry, and the ray flowers wilt (Fig. 1A–F).

Trupanea nigricornis females oviposited in the immature 'open' buds having a green, light-green, or yellow color (stages 2, 3, and 4; Figs. 1B–D). The flower heads that were suitable for oviposition had mean diameters ranging from 7.1–10.5 mm and mean lengths ranging from 4.3–7.4 mm (Table 1). The period of time that a flower head was suitable for oviposition was about 12 d: 8, 13, and 14 d in sites 3, 2, and 1, respectively. This period of suitability did not correspond to the entire bloom period of *E. farinosa*, as stems continued producing flower heads sequentially as long as there was enough moisture in the soil. The total period of flower-head development of *E. farinosa*, lasted ca. 40 d (5–7 weeks) in the field. The total period of development of *T. nigricornis* from oviposition to adult emergence from the mature heads ranged between

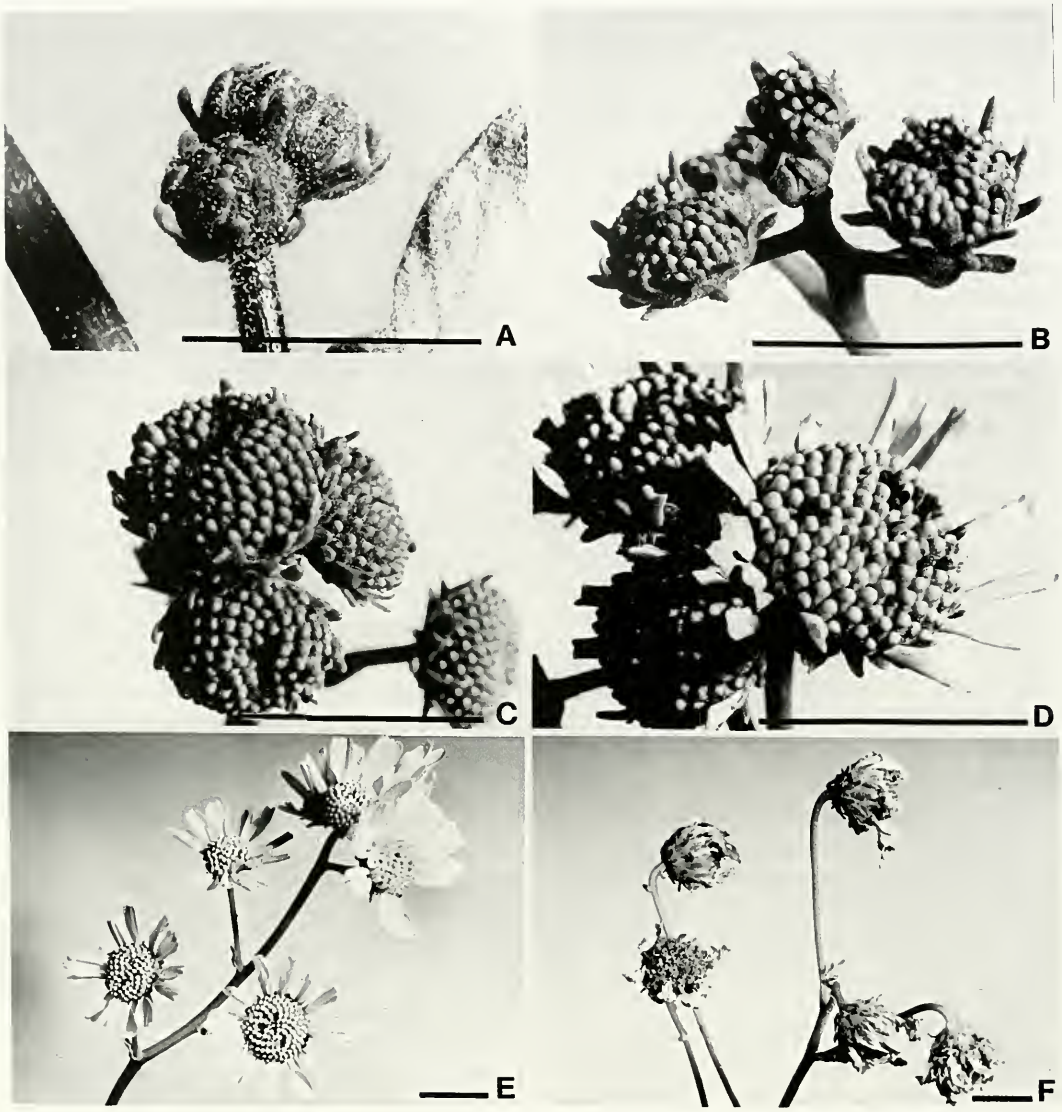

Fig. 1. Stages in flower head development of *Encelia. farinosa* (bar = 1 cm). A, 'Closed bud' stage. B, 'Open green bud' stage'. C, 'Open light-green bud' stage; D, 'Open yellow bud' stage. E, 'Open yellow bud' and 'blossom' stages. F, 'Post-blossom' stage.

25–30 d (ca. 4 weeks) in the field (Table 1).

Dissections of *E. farinosa* flower heads confirmed the results of the field phenology experiment. The flower heads that contained *T. nigricornis* eggs were open buds with green, light-green or yellow florets. These corresponded to the flower head stages 2, 3, and 4. The majority (53%) of the eggs (n = 100) were found in flower heads with light green florets; the rest (26% and 21%) were found in heads with green and yellow florets, respectively (Fig. 2). A correlation existed between flower head size and number of eggs laid per head. The flower heads with yellow florets were the largest and contained the greatest mean number of eggs (2.9) per head. They were closely followed by flower heads with light-

Table 1. Phenology of flower head development of *Encelia farinosa* at three locations in southern California showing the stages in which eggs of *Trupanea nigricornis* are found.

Days	Site 1		Site 2		Site 3		Head Stage[c]
	D[a] ± SE	L[b] ± SE	D ± SE	L ± SE	D ± SE	L ± SE	
1	2.3 ± 0.1	2.1 ± 0.1	3.4 ± 0.2	2.6 ± 0.2	3.2 ± 0.2	2.1 ± 0.1	GC −
3	---	---	3.8 ± 0.1	3 ± 0.1	4.4 ± 0.3	2.7 ± 0.1	GC −
5	3.9 ± 0.1	2.3 ± 0	---	---	---	---	GC −
7	4.8 ± 0.1	3 ± 0.1	6.1 ± 0.2	4 ± 0.4	5.9 ± 0.4	3.7 ± 0.3	GC −
10	6 ± 0.1	3.7 ± 0.1	**7.3 ± 0.5**	**4.3 ± 0.1**	6.8 ± 0.4	4.9 ± 0.4	OG +
15	**7.5 ± 0.1**	**4.4 ± 0.1**	**7.4 ± 0.3**	**4.7 ± 0.2**	**7.1 ± 0.4**	**5.3 ± 0.3**	OG +
18	---	---	**7.8 ± 0.3**	**4.9 ± 0.2**	**7.5 ± 0.4**	**6.3 ± 0.3**	OLG +
22	**9.1 ± 0.2**	**5.3 ± 0.2**	**9.3 ± 0.2**	**6.7 ± 0.3**	**8.9 ± 0.5**	**7.4 ± 0.6**	OLG +
28	**10.5 ± 0.2**	**7.2 ± 0.1**	10 ± 0.2	8.1 ± 0.3	8.7 ± 0.4	9.6 ± 0.4	OY +
33	10.8 ± 0.2	8.3 ± 0.1	9.9 ± 0.2	8.3 ± 0.3	8.6 ± 0.5	9.7 ± 0.2	BL −
40	10.3 ± 0.3	9.8 ± 0.2	9.4 ± 0.3	8.5 ± 0.2	8.4 ± 0.5	9.6 ± 0.2	PBL −

[a] Mean widest diameter and [b] mean length (from base of receptacle to tip of florets) ± standard error in mm of n = 10 flower heads for sites 1 and 3 and N = 20 heads for site 2.

[c] Flower head stages: closed green bud (CG); open green bud (OG); open light green bud (OLG); open yellow bud (OY); blossoming head (BL); post-blossoming head (PBL). Eggs found (+); no eggs found (−). The flower heads which contained eggs are marked in bold.

green florets that were intermediate in size and contained a mean of 2.5 eggs per flower head while the smaller heads with green florets contained a mean of 1.5 eggs per flower head (Fig. 2).

The development of wild sunflower heads, the host plant of *T. bisetosa*, was divided into six stages: (1) small 'closed bud' stage, in which the soft, straight, and short green bracts cover the immature florets; (2) advanced 'closed bud'-stage, in which the hard and curved bracts cover the immature florets; (3) 'open yellow bud' stage, in which the bracts are opened and the yellow florets remain immature, but the ray flowers are developing; (4) early 'blossom' stage, in which some of the florets are at anthesis, maturation of the florets has started gradually from the periphery to the center of the flower heads, and the ray flowers are fully developed; (5) advanced 'blossom' stage, in which the florets at the center of the flower head are at anthesis while the rest are at post-anthesis; (6) 'post-blossom' stage, in which the achenes are mature, the florets are at post-anthesis, and the ray flowers are wilting (Fig. 3A–F).

In the field, the females of *T. bisetosa* laid eggs in the immature flower heads of their host. However, unlike *T. nigricornis* females, which laid eggs in the open buds (stages 2–4) of *E. farinosa*, the females of *T. bisetosa* only oviposited in the closed green buds (stage 1) of wild sunflowers, and especially, in the very small closed buds (Fig. 3A). The early stages of closed buds apparently were preferred because they did not exude any resins when accidentally pierced by the female's ovipositor, and they also had softer, straighter, but short bracts (Fig. 3A) compared to the harder and curved bracts of the older closed buds (Fig. 3B). The areas between the elongate bracts that covered the immature florets formed direct channels into the center of the flower head into which females of *T. bisetosa* inserted their ovipositors. In the laboratory, three females out of 10 were observed to oviposit into the open green buds (stage 3) when not given a choice; this led to the death of two of these females because their ovipositors became trapped in the exuding resin. Cavender and Goeden (1982) also reported that in the insectary, *T. bisetosa* females accept-

Fig. 2. Stages in flower head development of *Helianthus. annuus* (bar = 1 cm). A, Small 'closed bud' suitable for oviposition by *T. bisetosa* females. B, Advanced 'closed bud' stage'. C, 'Open yellow bud' stage. D, 'Early blossom' stage. E, 'Late blossom' stage. F, 'Post-blossom' stage.

ed open flower heads, but that in the field they preferred closed flower heads.

The closed green buds that were suitable for oviposition by *T. bisetosa* females had diameters ranging from 5.0–9.8 mm (Table 2). The period of time that a sunflower head was suitable for oviposition in the field was ca. 5 d (Table 2), and much shorter than the suitability period of *E. farinosa* for oviposition by *T. nigricornis*. This suitability for egg laying covered only part of the entire flowering period of wild sunflowers, as some plants flowered throughout the year in southern California under favorable environmental conditions, e.g., mild frost-free winter and ample rainfall. The total period of development for wild sunflower heads was ca. 35–40 d in the field. Like *T. nigri-*

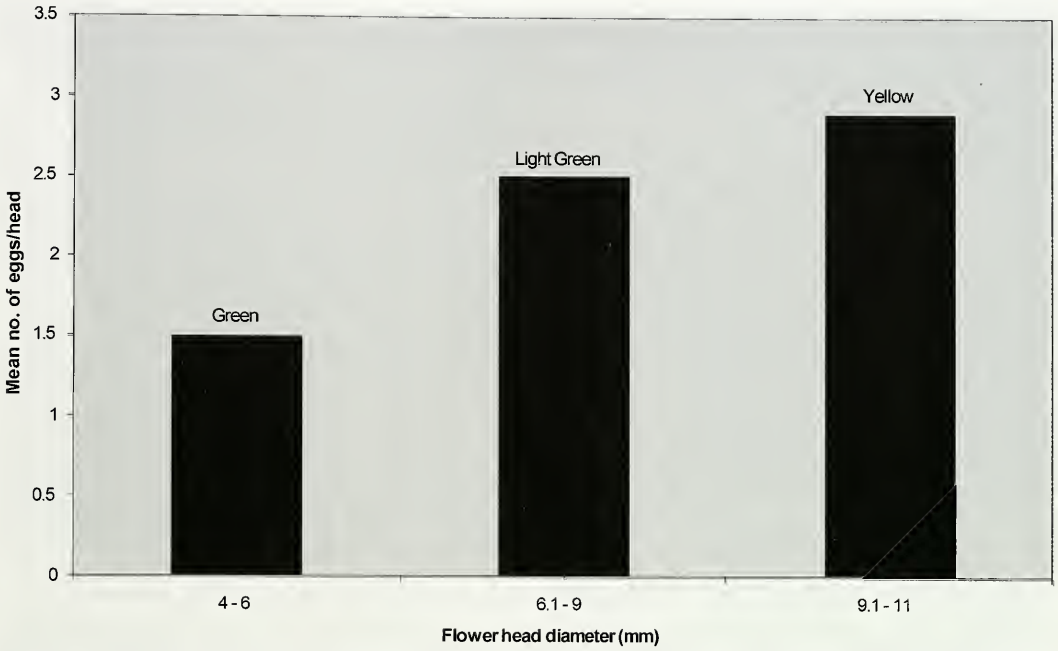

Fig. 3. Mean number of *Trupanea nigricornis* eggs found in three bud stages of *Encelia farinosa* heads (n = 100).

cornis, the period of development of *T. bisetosa* from egg to adult ranged from 30–35 d (ca. 4–5 weeks).

Dissections of field-collected sunflower heads confirmed these findings on phenology. The flower heads that contained *T. bisetosa* eggs (n = 100) were all closed buds with straight bracts (stage 1, Fig. 3A). The mean diameter of these buds ranged from 5.0–7.8 mm and their mean length ranged from 6.5–8.5 mm (Table 2). The dissected buds contained mainly 3–5 eggs as previously reported by Knio et al. (1996b).

Table 2. Phenology of flower head development of *Helianthus annuus* showing the stages suitable for egg laying by *Trupanea bisetosa*.

Days	D[a] ± SE	(Range)	L[b] ± SE	(Range)	Head Stage[c]
1	5 ± 0.1	(4.5–5.8)	6.5 ± 0.1	(5–7.8)	CG +
3	6.3 ± 0.1	(5.7–7)	7.6 ± 0.2	(6.7–9.7)	CG +
5	7.8 ± 0.1	(7–8.8)	8.5 ± 0.1	(7.4–9.8)	CG +
7	10.4 ± 0.2	(8.9–12.8)	10.6 ± 0.2	(9–12.5)	CG −
10	16.7 ± 0.4	(13.5–19.8)	13.6 ± 0.6	(9.5–17.6)	OG −
15	17.6 ± 0.5	(14.2–21)	13 ± 0.7	(8.7–18)	OLG −
18	20.8 ± 0.5	(16.7–24)	12.9 ± 0.4	(9.5–16)	OY −
22	22.4 ± 0.3	(19.3–24.1)	12.7 ± 0.3	(10.6–16.2)	OY −
28	24.3 ± 0.6	(20.1–29.4)	16.6 ± 0.4	(13.3–19.5)	BL −
33	25.2 ± 0.4	(20.5–27)	20.5 ± 0.4	(18.8–22.1)	BL −
40	25.9 ± 0.6	(20.8–29.2)	20.3 ± 0.4	(17.2–23)	PBL −

[a] Mean widest diameter and [b] mean length (from base of receptacle to tip of florets) ± standard error in mm of n = 20 flower heads.

[c] Flower head stages: closed green bud (CG); open green bud (OG); open light green bud (OLG); open yellow bud (OY); blossoming head (BL); post-blossoming head (PBL). Eggs found (+); no eggs found (−).

Similar to *T. bisetosa*, the window for oviposition suitability was narrow and restricted to a short phase, 'unopened buds', in *Trupanea conjuncta* (Adams), *Tephritis dilacerata* Loew and *T. formosa* Loew (Goeden 1987; Berube 1978a, b). *Trupanea conjuncta* females only laid eggs in the small green buds of their host, *Trixis californica* Kellog (Asteraceae) and this stage lasted about 5 d (Goeden 1987). *Tephritis dilacerata* females deposited their eggs in the closed buds of *Sonchus arvensis* L. (Asteraceae) and weaved their ovipositors slowly in between the bracts in order to avoid piercing the host tissues and releasing sticky latex (Berube 1978a). Both *T. dilacerata* and *T. formosa* timed oviposition to coincide with the stage of unopened buds when they were at their maximum growth as this stage was the most suitable for gall induction by the young larvae (Berube 1978b). On the other hand, similar to *T. nigricornis*, the suitability period for oviposition by the tephritids, *Tephritis bardanae* (Schrank) and *Cerajocera tussilaginis* (F.) on *Arctium minus* (Hill) Bernh. (Asteraceae) was 10–11 d; however, there was no overlap in the oviposition suitability periods as *T. bardanae* oviposited early in the smaller unopened buds while *C. tussilaginis* followed a late attack strategy and deposited eggs in the pre-flowering heads. Like *T. nigricornis* and *T. bisetosa*, these flies never laid eggs in flower heads that had started to flower (Straw 1989). Females of *Chaetostomella undosa* (Coquillett) also oviposited in the closed and open buds of their host, *Cirsium cymosum* (Greene) J. T. Howell (Asteraceae), but like *T. nigricornis*, they showed a preference to open buds (Steck 1984).

In selecting heads suitable for oviposition, female tephritids seem to compare their body size and length of their oviscape to flower head dimensions (Straw 1989). The length of the oviscape of several *Urophora* species have been found to be correlated with the diameter of flower heads exploited, implying "evolutionary responses of these phytophages to a particular structure of the host plants"(Zwölfer 1987). Moreover, in addition to the length of the ovipositor, the aculeus tip bears a number of sensilla that are used to guide the female during oviposition. In the following section, we examine the ultrastructure of the ovipositors of *T. nigricornis* and *T. bisetosa* in order to better understand the differences in their oviposition behavior.

Ultra-structure of the ovipositors.—The external anatomy of the ovipositors of *T. nigricornis* consists of the modified seventh abdominal segment or oviscape (syntergosternite 7), an eversible membrane, and an aculeus (Norrbom and Kim 1988, White et al. 1999) composed of three, long, parallel processes, two ventral and one dorsal (Stoffolano and Yin 1987), which are the eighth sternites or ventral flaps, and the eighth tergite, respectively (White et al. 1999) (Fig. 4C, F).

The oviscape of *T. nigricornis* is conical in shape and heavily sclerotized (Fig. 4A). It measured (from tip to base on the ventral side) 1.05 ± 0.014 (range: 0.89–1.16) mm in length (n = 25).

The eversible membrane (0.21 mm as greatest width) of *T. nigricornis* also is heavily sclerotized (Fig. 4B). It is covered with acanthae, scale-like, cuticular projections (Fig. 4D–E). When the ovipositor is retracted only the oviscape is apparent (Fig. 4A–B); the rest of the ovipositor, including the eversible membrane and aculeus, is housed within the seventh abdominal segment.

The retractable eversible membrane connects the oviscape to the aculeus (Fig. 4A–C). The eversible membrane is visible only when the ovipositor is extended. It is covered with acanthae that point anteriad. The acanthae gradually increase in size from the tip of the

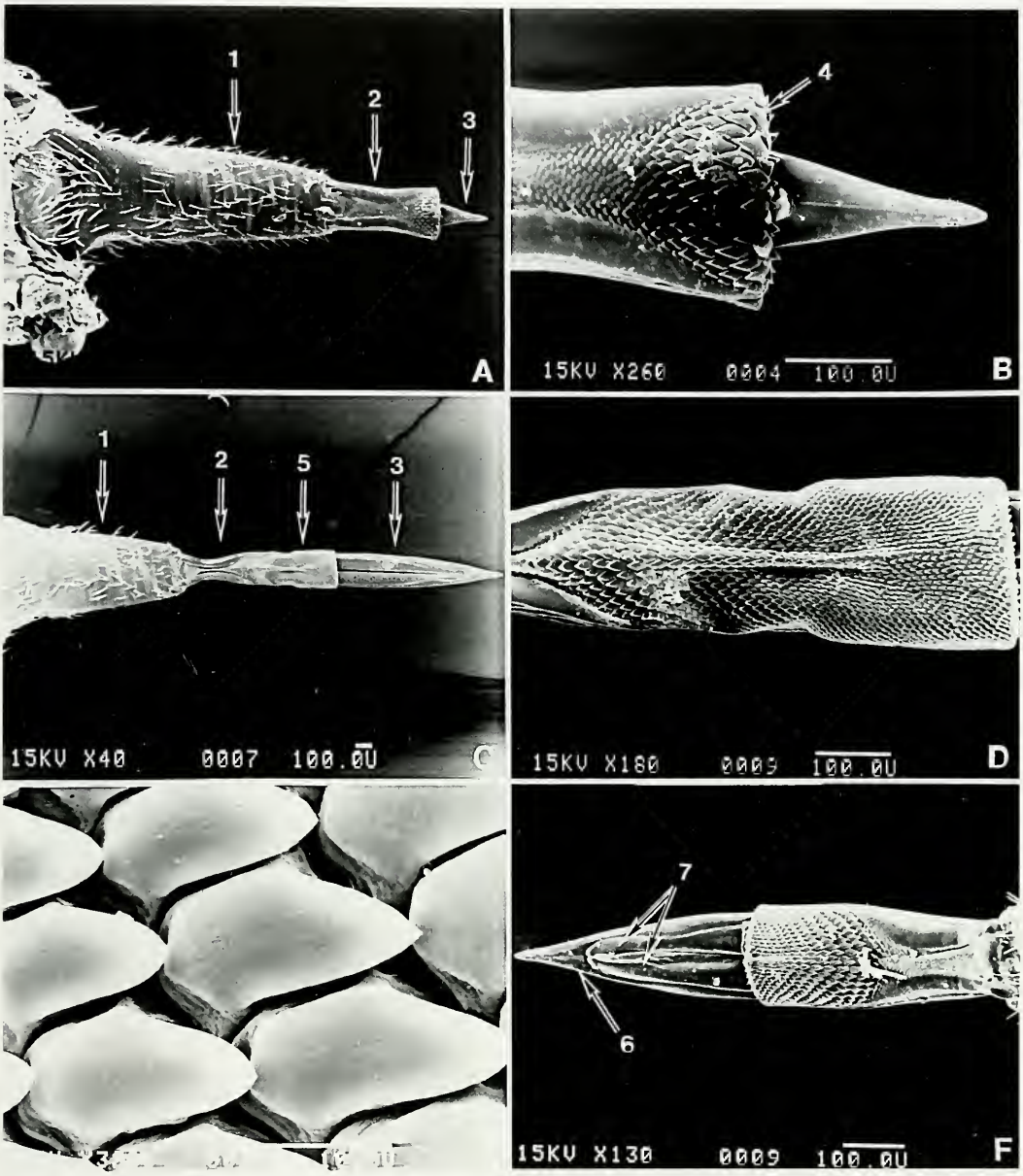

Fig. 4. Scanning electron micrographs of the ovipositor of *Trupanea nigricornis*. A, Ventral view of the ovipositor showing (1) the oviscape, (2) the eversible membrane, and (3) the tip of the aculeus. B, Ventral view of the eversible membrane showing (4) the pointed acanthae. C, Ventral view of the extended ovipositor showing (1) the oviscape, (2) basal region and (5) distal region the eversible membrane, and (3) the aculeus. D, The eversible membrane. E, Pointed acanthae on the eversible membrane. F, Ventral view of the basal part of the eversible membrane, and the aculeus showing (6) the eighth tergite and (7) the ventral flaps or eighth sternites.

distal region to the base of the basal region of the eversible membrane (Fig. 4D). These acanthae are sharply pointed in *T. nigricornis* (Fig. 4E).

The aculeus of *T. nigricornis* has a sharply pointed apex. It bears two ventral sclerites (eighth sternites) and a dorsal sclerite (eighth tergite) (Fig. 4F).

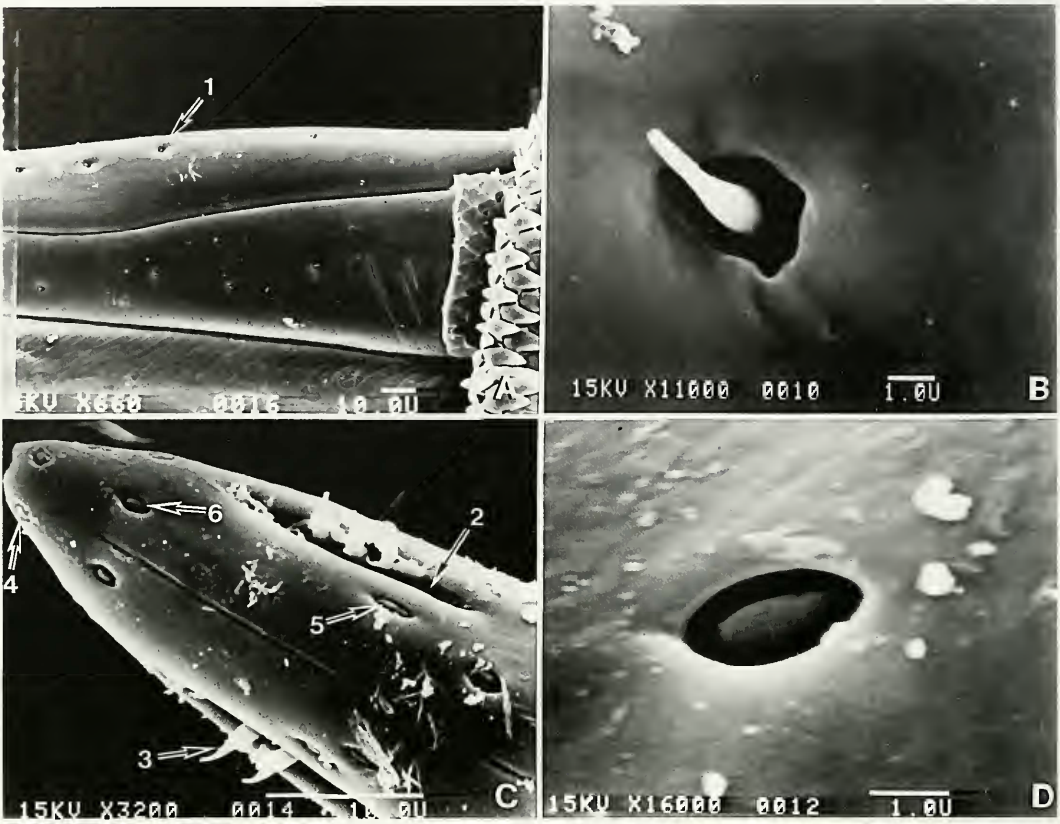

Fig. 5. Scanning electron micrographs of the ovipositor of *Trupanea nigricornis*. A, Lateral aspect of the dorsal process with the row of (1) hair-like sensilla. B, Hairlike sensillum on the dorsal process. C, Apical region of the aculeus showing (2) the two ventrolateral grooves with (3) the elongated sensilla (three per groove), (4) the shallow ampulliform sensilla, (5) the ellipsoidal ampulliform sensilla, and (6) the central ampulliform sensilla. D, Central ampulliform sensillum sunken in an oval socket.

The ventral sclerites appear as two parallel, elongate structures with blunt ends on the ventral side of the ovipositor. They are shorter than the dorsal sclerite and only visible when the ovipositor is extended. The ventral sclerites, which are joined by a flexible, median and infolded membrane, do not completely meet (Stoffolano and Yin 1987, Zacharuk et al. 1986); a ventral groove extends between them and terminates in the cloaca, the common opening to the reproductive and digestive tract. The dorsal sclerite (eighth tergite) measured 0.14 mm at greatest width (n = 4) and 1.27 mm in length (n = 4) (Fig. 4F). It bears a ventrolateral row of hairlike sensilla (Fig. 5A) that have blunt tips. The sensilla are surrounded by a shallow depression (Fig. 5B). Such sensilla also occur on the ventral sclerites (Fig. 5B). These hairlike sensilla are similar to those described for the apple maggot (Stoffolano and Yin 1987). They are numerous (50–60) on the dorsal and (11–12) ventral sclerites of the apple maggot ovipositor; they were identified as mechanoreceptors innervated by a single neuron, and were not associated with chemoreception (Stoffolano and Yin 1987).

The tip of the dorsal sclerite (eighth tergite) of the aculeus of *T. nigricornis* bears different types of sensilla that show

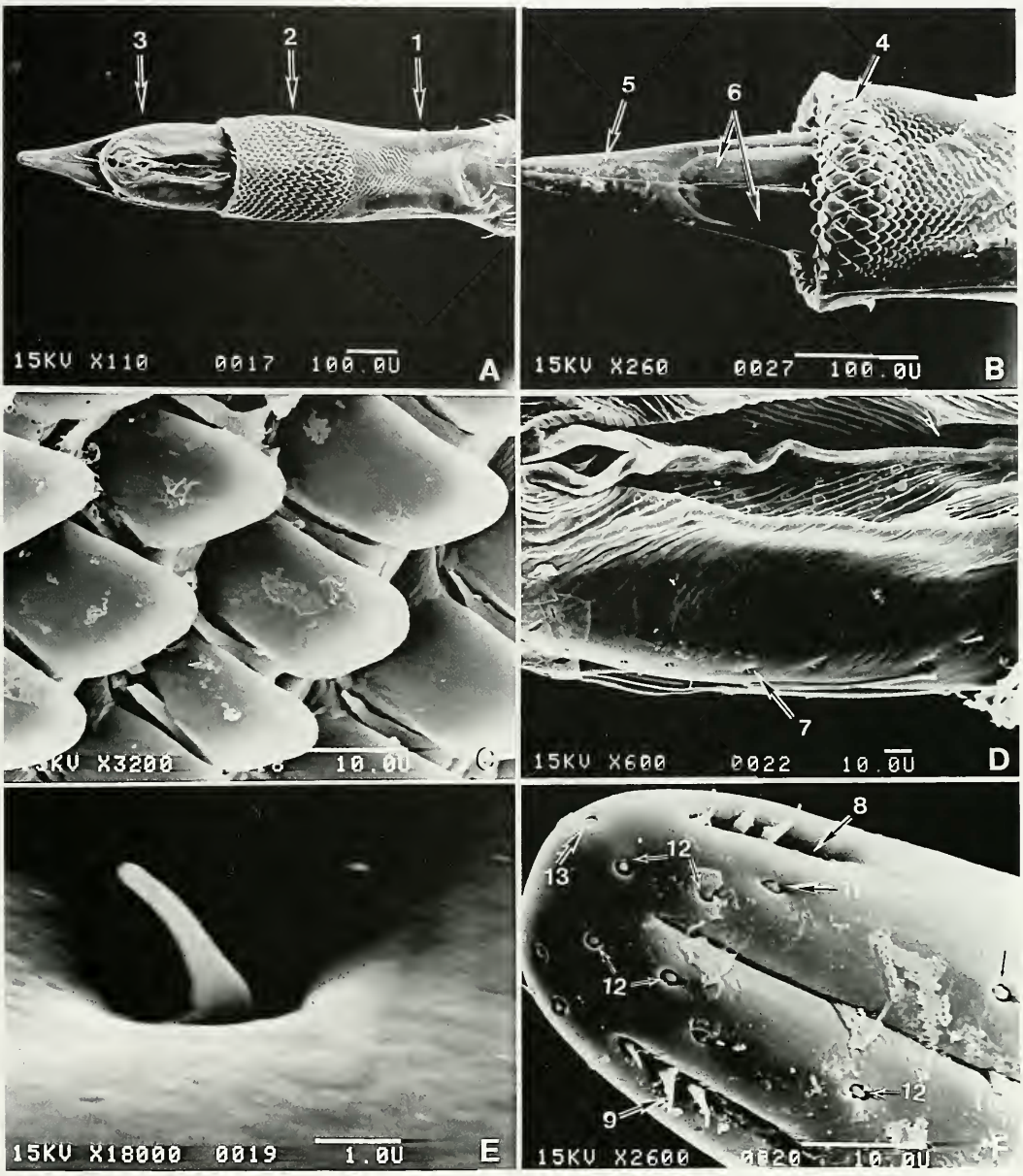

Fig. 6. Scanning electron micrographs of the ovipositor of *Trupanea bisetosa*. A, Ventral view of the ovipositor showing (1) the basal region, (2) the distal region of the eversible membrane, and (3) the aculeus. B, Ventral view of the eversible membrane covered by (4) acanthae, and the aculeus showing the (5) eighth tergite and (6) eighth sternites (ventral flaps). C, Rounded acanthae on the eversible membrane. D, Lateral aspect of the eighth tergite of the aculeus showing the row of (7) hairlike sensilla. E, Hairlike sensillum contained in a shallow socket. F, Apical region of the aculeus showing the rounded tip of the ovipositor, (8) the two lateroventral grooves with (9) the three pairs of elongated sensilla, (11) the shallow ampulliform sensilla, (12) the central ampulliform sensilla, and (13) the ellipsoidal ampulliform sensilla.

bilateral symmetry (Fig. 5C). There are two ventrolateral grooves, each located distad of the ventral sclerites. The ventrolateral grooves bear three pairs of elongated sensilla that probably are chemoreceptors based on comparative

morphology (Fig. 5C). The dorsal sclerite also bears several types of ampulliform sensilla. Close to the medial line ventrally dividing the dorsal sclerite are two pairs of ampulliform sensilla that appear to be similar morphologically, but may have different functions. These are short sensilla, referred to as central ampulliform sensilla, sunken in deep, oval sockets (Fig. 5C–D). Another pair of elongated ampulliform sensilla sunken in deep, ellipsoidal sockets is located closer to the ventrolateral grooves than the medial line (Fig. 5C). The lateral sides of the ovipositor tip are also covered by several shallow ampulliform sensilla located in shallow, oval sockets (Fig. 5C).

The gross anatomy of the ovipositor of *T. bisetosa* is similar to that of *T. nigricornis*. It consits of the oviscape, eversible membrane, and aculeus (Fig. 6A). The oviscape is conical and heavily sclerotized (Fig. 6A). Its length is close to that of *T. nigricornis*, measuring 1.08 ± 0.02 (range: 0.92–1.28) mm (n = 25) using light microscopy. The eversible membrane is covered with acanthae that point anteriad; however, contrary to the pointed acanthae of *T. nigricornis*, the acanthae in *T. bisetosa* have rounded tips (Fig. 6C).

The aculeus in *T. bisetosa* also is composed of a long dorsal sclerite and two shorter ventral ones (eighth sternites). Like *T. nigricornis*, the dorsal sclerite of *T. bisetosa* measured 0.15 mm (n = 2) at its greatest width and 1.28 mm (n = 3) in length, and is covered with hairlike sensilla laterally. Each hairlike sensillum is surrounded by a shallow circular socket (Fig. 6D–E). The tip of the aculeus has a rounded shape in *T. bisetosa*, as opposed to the pointed aculeus tip in *T. nigricornis* (Fig. 6F vs. Fig. 5C). Similar to *T. nigricornis*, the apex of the aculeus of *T. bisetosa* bears two ventrolateral grooves with three pairs of elongated sensilla (Fig. 6F), and a pair of ellipsoidal ampulliform sensilla near the ventrolateral grooves (Fig. 6F). Unlike *T. nigricornis*, which has two pairs of central ampulliform sensilla, *T. bisetosa* has three pairs of central ampulliform sensilla sunken in deep, oval sockets close to the medial line of the dorsal sclerite (Fig. 6F).

The gross structure of the ovipositors of *T. nigricornis* and *T. bisetosa* is close to that described for other tephritids (Marchini and Wood 1983, Stoffolano and Yin 1987, Zacharuk et al. 1986). The apices of the ovipositors of *Rhagoletis pomonella* (Walsh) (Stoffolano and Yin 1987), *Urophora affinis* Frauenfeld (Zacharuk et al. 1986), and *Ceratitis capitata* (Wied.) (Marchini and Wood 1983) have two types of mechanosensilla: hairlike and campaniform sensilla, both sunken in shallow pits and lacking a pore to the outside. These hairlike sensilla are similar to those occurring laterally on the dorsal sclerite of the aculeus of *T. nigricornis* and *T. bisetosa*. The campaniform sensilla at the apex of the aculeus of *R. pomonella* and *U. affinis* appear similar to the shallow ampulliform sensilla described for *T. nigricornis* and *T. bisetosa*. Such mechanoreceptors have been found to function in monitoring the hardness of the fruit surface; guiding the ovipositor during piercing and penetrating the fruit pulp; and monitoring the ovipositor position in the fruit, the egg passage, physical contact with the male during copulation, and contact with the fruit during post-ovipositional dragging of the ovipositor (Stoffolano 1989, Stoffolano and Yin 1987). The campaniform sensilla found at the very tip of the aculeus most probably monitor the amount of stress imposed on the cuticle during penetration of the flower head (Zacharuk et al. 1986). The hairlike sensilla or 'short trichoid hairs' probably monitor the depth of penetration of the aculeus and its movement in the flower head (Zacharuk et al. 1986).

The apex of the ovipositor of *T. nigricornis* and *T. bisetosa* is also similar to that of *R. pomonella* in having two ventrolateral grooves, each with three elongated sensilla, identified as chemosensilla (Stoffolano and Yin 1987). Similar uniporous chemosensilla located in ventrolateral grooves are also found at the tip of the aculeus of *C. capitata*, but each groove has five sensilla (Marchini and Wood 1983). These chemoreceptors are also found in *U. affinis*; however, there are just two on each side of the aculeus tip and each is located in an individual deep ventrolateral socket (Zacharuk et al. 1986). These serve as mechano-chemosensilla (Zacharuk et al. 1986). In addition to these, the aculeus tip of *U. affinis* and *R. pomonella* contains a fourth type of uniporous mechano-chemosensilla: one pair on each side of the aculeus tip located distally and outside the ventrolateral grooves (Stoffolano and Yin 1987, Zacharuk et al. 1986).

Each of the uniporous chemosensilla in the ventrolateral grooves is associated with three or four chemosensitive neurons and one mechanosensillum (Stoffolano and Yin 1987, Girolami et al. 1986). Possible functions suggested for these chemosensilla are to locate suitable hosts, to assess host suitability and quality, to locate a suitable oviposition site, and to detect conspecific flies or oviposition-deterrent pheromones (Stoffolano 1989, Stoffolano andYin 1987). Zacharuk et al. (1986) noted that since the chemosensilla are located on the ventrolateral aspect of the ovipositor tip, "only the tip of the ovipositor blade 'tastes' or 'smells'...and the egg is deposited just above or at the level of the last 'taste'." Using electrophysiological techniques, it has been found that the contact chemosensilla at the apex of the ovipositor of *R. pomonella* responded to stimulation by various substances like glucose, fructose, and malic acid (Girolami et al. 1986). Also, *R. pomonella* detected the addition of these chemicals to fruits by probing, and they laid more eggs when the fruits were treated with substances like glucose and malic acid. It was also found that the destruction of the chemosensilla affected the ability of the females to discriminate between treated and control fruits. Thus, "the presence of more than one chemosensillum per sensilla may provide the fly with an input that can be used by the fly to discriminate between various types of fruit and/or fruit quality" (Girolami et al. 1986).

Similar to *T. nigricornis* and *T. bisetosa*, the eversible membranes of *R. pomonella* and *Anastrepha* spp. are covered with acanthae, minute 'teeth' or scales (Stoffolano and Yin 1987, Norrbom and Kim 1988). The acanthae 'may hold the base of the aculeus in place during oviposition' (Norrbom and Kim 1988), or may anchor the female's abdomen during the process of fruit penetration (Stoffolano and Yin 1987).

The differences in the shape of the acanthae and the aculeus tip, i.e., pointed in *T. nigricornis* versus rounded in *T. bisetosa*, appear to be related to the different host plants they use and to their specific oviposition behavior. Since females of *T. bisetosa* oviposit in the flower heads of wild sunflowers which exude copious resins when pierced, both the rounded acanthae and rounded aculeus tip allow them to deposit their eggs superficially without injuring the plant tissues and becoming caught in the resins. On the other hand, the hosts of *T. nigricornis* do not exude much resin, and the females lay their eggs deeper in the flower heads by inserting the posterior pole of the egg into the plant tissues (Knio et al. 1996b). In that case, the pointed acanthae may help the females to better anchor themselves and lay the eggs closer to the achene, and the pointed tip of the ovipositor facilitates

insertion of the eggs of *T. nigricornis* into the plant tissues. Therefore, it seems that the ovipositor of *T. bisetosa* is adapted for shallow penetration of the plant tissues while that of *T. nigricornis* is adapted for deeper penetration and piercing of host tissues. Moreover, the additional pair of central ampulliform sensilla at the apex of the aculeus observed in *T. bisetosa* might be essential in detecting the suitability of wild sunflower heads for oviposition as well as sensing the position of the florets during egg insertion to avoid piercing the plant tissues.

In conclusion, the basis of host specificity in these closely related and sympatric tephritids appears to be associated with female behavior and related to the biophysical features of the host plants. The specialization on wild sunflowers by *T. bisetosa* required behavioral and morphological adaptation to overcome the problem of hard bracts and resins. Zwölfer (1983, 1987) noted that host adaptation in tephritids frequently involved adaptive changes that are reflected in morphological traits, such as the ovipositor tip and length. "These integrated differentiation processes on the ecological, biological, physiological, and morphological level are consequences of the co-evolution of the tephritid taxon with a given plant taxon" (Zwölfer 1983).

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